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C-Banding of Chromosomes from Three Established Marine Fish Cell Lines

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16. ABSTRACT

Many fish karyotypes possess a large group of morphologically similar chromosomes. The authors describe a technique developed through modification of a C-banding procedure that produces distinct bands and thus allows identification of the heterochromatic regions of individuals pairs of fish chromosomes. The technique has been applied to three established marine fish cell lines.

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improved the spreading and eliminated a certain amount of cellular overlap.

The advantage of this modification of the scale epithelium technique of Denton and Howell (1969) is that cells that are at different stages of mitosis are blocked at metaphase and yield a higher number of mitotic spreads with analyzable chromosomes (Fig. 1). In this technique, the colchicine treatment is done in vitro while the cells are in a physiological and isotonic environment. Chromosome spreads that have not been given the colchicine treatment will usually be in different stages of mitosis and the number of analyzable spreads is reduced. An additional advantage of this technique is that the amount of handling of the fish is kept at a minimum and the fish is not sacrificed. This is important in environmental, reproductive, and genetic studies where the fish need to be monitored over a long period of time without significantly affecting the animal and still be able to analyze the chromosomes rapidly. A disadvantage in using scale epithelium is that the scales inherently have a low mitotic index. Therefore, the in vitro colchicine treatment will enhance the number of analyzable chromosomal spreads found in the scale epithelium. This technique has been used successfully in both marine and fresh water fishes.

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C-BANDING OF CHROMOSOMES FROM THREE ESTABLISHED MARINE FISH CELL LINES.—Differential Giemsa staining of the constitutive heterochromatin in human chromosomes has become a useful and accepted technique (Arrighi and Hsu, 1971). Such a staining technique could be of great value when applied to the chromosomes of other organisms, especially those with numerous morphologically identical chromosomes. Many fish karyotypes possess a large group of morphologically similar chromosomes. A technique has been developed through modification of a C-banding procedure that produces distinct bands and thus allows identification of the heterochromatic regions of individual pairs of fish chromosomes. This technique has been applied to three established marine fish cell lines. C-banding has been reported on several trout species (Abe and Muramoto, 1974; Zenzes and Voiculescu, 1975; Thorgaard, 1976); however, none of these studies employed established cell lines.

Materials and methods.—The slides used were prepared from fish fibroblast cultures by the method of Sumner et al. (1971). C-bands were

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Fig. 1. C-banded karyotype from spleen fibroblasts of the silver perch at pass 36. The arrow denotes the pair with two C-bands present.

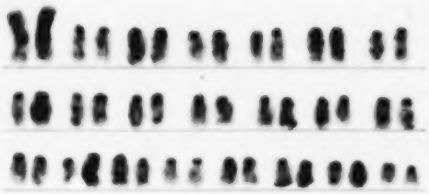

Fig. 3. C-banded karyotype from fin fibroblasts of the blue striped grunt at pass 182.

produced using a modification of the procedure used for human leukocyte cultures described by Howard et al. (1973). The technique was changed by increasing the incubation time from 24 to 48 hours and staining the slides for 15 minutes rather than 9 minutes in the Giemsa stain solution.

This procedure was used on slides prepared from cultured spleen fibro blasts of the silver perch (*Bairdiella chrysura*) (Ellender et al., 1978), from cultured fin fibroblasts of the sheepshead (*Archosargus probatocephalus*) (Law et al, 1978) and the salt water bluestriped grunt (*Haemulon sciurus*) (Clem et al., 1961; karyotype characterization by Regan et al., 1968). All cultures were maintained by the tissue culture facility of the Department of Microbiology (University of Southern Mississippi) using standard procedures.

Results.—Representative karyotypes from these three marine fish lines are shown in Figs. 1, 2 and 3. C-banding characteristically caused a diffuse appearance of the chromosomes with the heterochromatin (C-bands) staining very dark. The silver perch chromosomes (Fig. 1) have a C-band present in the centromere region of each telocentric with 1 pair having 2 bands. The sheepshead chromosomes (Fig. 2) are more diverse having both telocentric and acrocentric chromosomes with 1 large metacentric. The first 3 acrocentric pairs show both a centromeric and short arm band with pair 1 having what appears to be satellites. Pairs 10, 11, and 12 also appear to have heterochromatic short arms; other pairs (4, 18, 19, 22) have nonheterochromatic short arms. Greenbaum et al. (1978) made this same observation in deer mice; its significance is not yet clear. All other chromosomes have at least 1 C-band located at the centromere. The grunt chromosomes (Fig. 3) are all telocentrics with the exception of a large metacentric pair. The C-band pattern is found primarily in the centromeric area with lightly staining areas along the long arm allowing better matching of the pairs.

Fig. 2. C-banded karyotype from fin fibroblasts of the sheepshead at pass 51.

Discussion.—Due to the high number and small size of the chromosomes of many fish species, a C-banding technique is an important aid in distinguishing between similar appearing chromosomes. Other applications could include its being used to define intraspecies chromosomal differences which presently is not always possible (Manna and Prasad, 1974; Abe and Muramoto, 1974; Thompson et al., 1978). In fish species with larger chromosomes, it can be used to work out taxonomic relationships and evolutionary patterns. Another promising application is its usefulness in detecting chromosomal rearrangements since such rearrangements are very prevalent in long-term tissue culture lines.

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